AN EXPERIMENT IN USING MICROCUVETTES.

MANUFACTURE OF THIN GLASS STRIPS, PLATES, AND COVER GLASSES BY DRAWING SHEET GLASS

B. V. Perfil'yev, D. R. Gabe

Translation of "Opyt primeneniya mikrokyuvet"; "Izgotovleniye tonkikh steklyannykh lent, plastin i pokrovnykh stekol rastyagivaniyem listovogo stekla".
In: Capillary Methods of Investigating Microorganisms. Izdatel'stvo Akademii Nauk SSSR, Moscow/Leningrad, pp. 213-221 and 463-470, 1961.

NASA TT F-10,728

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON D.C. FEBRUARY 1967

AN EXPERIMENT IN USING MICROCUVETTES

A description of a special inovulation chamber for implanting isolated microbe cells is given. The microcuvettes are designed for growing cultures and observation under a microscope. The second article reviews plate glass manufacture in the USSR, and discusses the fabrication of microscope cover glasses.

Owing to the cover thinness of their inner folds, the microcuvette types /213* described here are suitable for all the tasks listed at the beginning of the chapter. We used them chiefly for growing microcultures from single cells obtained with a capillary microselector.

As mentioned above (see Chapter 6), the cell isolated by means of the microselector may be dropped into a test tube with a liquid or solid substrate. This is the simplest procedure, but in such "blind" inoculation the result will be clear only when the microorganism vigorously multiplies, and a sufficiently large colony forms on the agar, or a thick suspension/in the liquid substrate. The first most interesting, and least studied, initial stages in colony development are entirely inaccessible to observation. In order to make the study of the isolated cell possible and practically convenient from the very start of its germination or division, we shall perform the inoculation in the above-described microcuvettes.

Circular microcuvettes are suitable for the seeding of just a single cell. Depositing 6 to 9 individual drops of substrate into an elongated rectangular cuvette, we may implant the same number of isolated single cells.

A single-celled culture of fungi (e.g., *Penicillium*) may be kept for an indeterminate length of time in individual round microcuvettes, The colonies must be transplanted from the composite elongated microcuvettes before the start of sporulation in the microculture.

Isolated cells are conveniently inoculated in microcuvettes by means of a transplantation chamber which we specifically constructed.

The latter is a heavy, and therefore steady, metal case, a general view of which is given in Figure 97. The width of the internal opening is somewhat greater than that of the internal folds of the elongated cuvette, which makes /214 it possible to move the latter lengthwise in the chamber. The lengthwise motion is accomplished with two glass blocks which almost entirely cover the side slits in the chamber. In the center of the inoculation chamber roof, a square hole is cut out and covered with an ordinary cover glass, which is held in place by a pair of springs.

There are two or three inlets into the chamber. One is in the front part in the form of a slanting slot in the side wall pointing under the "window" into the center of the cuvette; the second leads from above to the same point in the form of an inclined tubular inlet covered with a cylindrical cap. For different

*Numbers in the margin indicate pagination in the original foreign text.

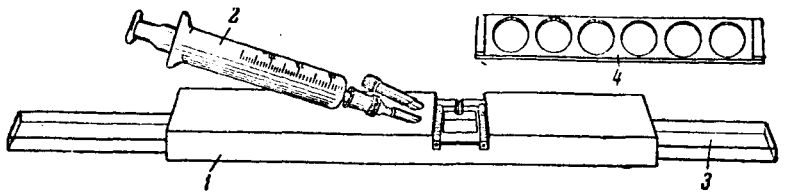

Figure 97

Inoculation Chamber for Implanting Isolated Single Microbe Cells Into the Microcuvette

1 - Metallic housing of the chamber; 2 - Syringe with additional liquid substrate, 3 - Glass block for moving the microcuvette under the chamber "window"; 4 - Strips with nests for moving several round microcuvettes into the implantation chamber at the same time.

supplementary operations, a third inlet may be made alongside and like the second, also directed toward the center under the chamber "window".

When oblong (rectangular) microcuvettes are used, a number of drops (6 to 9) of a heated sterile agar substrate (1 - 1.5%) are deposited with a fine pipette on the inner surface of the smaller fold. Transparent media are desirable for this purpose.

While the substrate is being deposited upon it, the microcuvette surface /215
may be protected from external infection by the external fold, the lid of the Petri dish, or the cover of the inoculation chamber. In the last case, the cover of the internal microcuvette fold is removed, and the leaf is quickly covered with the inoculation chamber. The glass blocks are used to move one of the ends of the microcuvette underneath the chamber "window." The cover glass is shifted slightly toward the rear wall, and the tip of the pipette with the melted agar is inserted into the slanting slot which is opened. While the cuvette fold is moved, one drop of substrate after another is deposited on it. Then the chamber is removed, and the microcuvette is quickly covered with the external fold previously sterilized in a flame.

The microcuvettes thus prepared are stored in Petri dishes, the bottoms of which must be covered with moist filter paper. Moreover, slides must be placed under the microcuvettes in order to avoid possible absorption of water from the surface of the filter paper into the microcuvette (Figure 98).

The microcuvette is removed from the moistened Petri dish only at the moment of inoculation--i.e., after successful infection of the capillary core of the microselector--and is then put under the inoculation chamber.

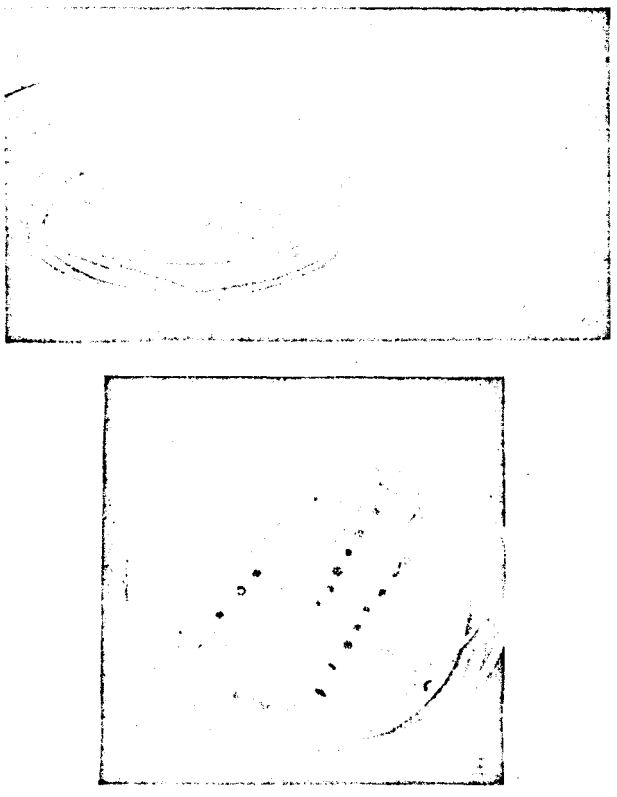

Figure 98

Microcultures in Cuvettes With Rectangular Folds

A - Petri dish with rectangular cuvettes prepared for inoculation of isolated microbe cells; B - A number of *Torula nigra* microcultures cultured in microcuvettes from single cells isolated with a microselector.

To protect the substrate drops from the extremely undesirable effect of drying out, which occurs rather quickly outside the Petri dish, a sheet of moist filter paper must be put under the inoculation chamber, which is set on a glass which is flame-sterilized or swabbed with alcohol. Into one of the lateral tubular inlets, previously protected with caps, we insert a syringe or pipette with sterile liquid substrate. For *Penicillium sp.*, for example, the substrate may be a sugar solution, Czapek's solution, brewer's mash, or simply water.

After the fragment of the capillary core with the cell has fallen into the receiver, a drop of liquid 0.5 to 1.0 mm in diameter is deposited by the syringe or micropipette on the next agar drop in line.

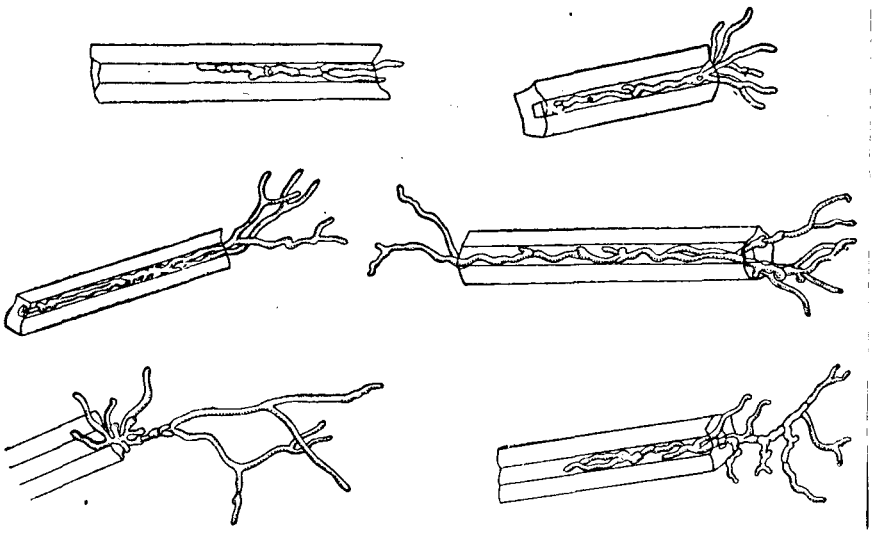

Figure 99

Microcuvette Germination of *Penicillium* Spores Isolated by a Microselector (40 Hours After Inoculation; Magnification 40 x 15).

The cover glass is moved from the slot, the receiver is introduced into the inoculation chamber, and the core fragment is dropped into the substrate drop (see Chapter 6, Section 4).

After implantation, the cuvette must be closed by the external fold, turned up by the thin lid, and examined under the microscope. Owing to the thinness of the internal fold covering and the slight depth of the agar drops, the object is quite accessible for high magnifications under the microscope. With an ocular of 10 or 15, and an objective of 40 or 60, not only isolated conidia, but also bacterial cells are clearly visible in the channel of the core fragment if the latter lies with its face upwards. This fragment must, of course, be determined in the microcuvette with weak illumination.

When separate, round microcuvettes are used for the seeding, they must be put into a holder in the shape of a rectangular plate with round nests in order to be conveniently moved in the inoculation chamber. This holder is filled with microcuvettes and placed in the inoculation chamber. The covers must be gradually taken off the microcuvettes as they are moved under the roof of the inoculation chamber.

After the microcuvette has been examined and the drops determined in which an isolated cell--or in any case the segment of the capillary core--is actually observed the microcuvette is returned to the moistened Petri dish and set to mature.

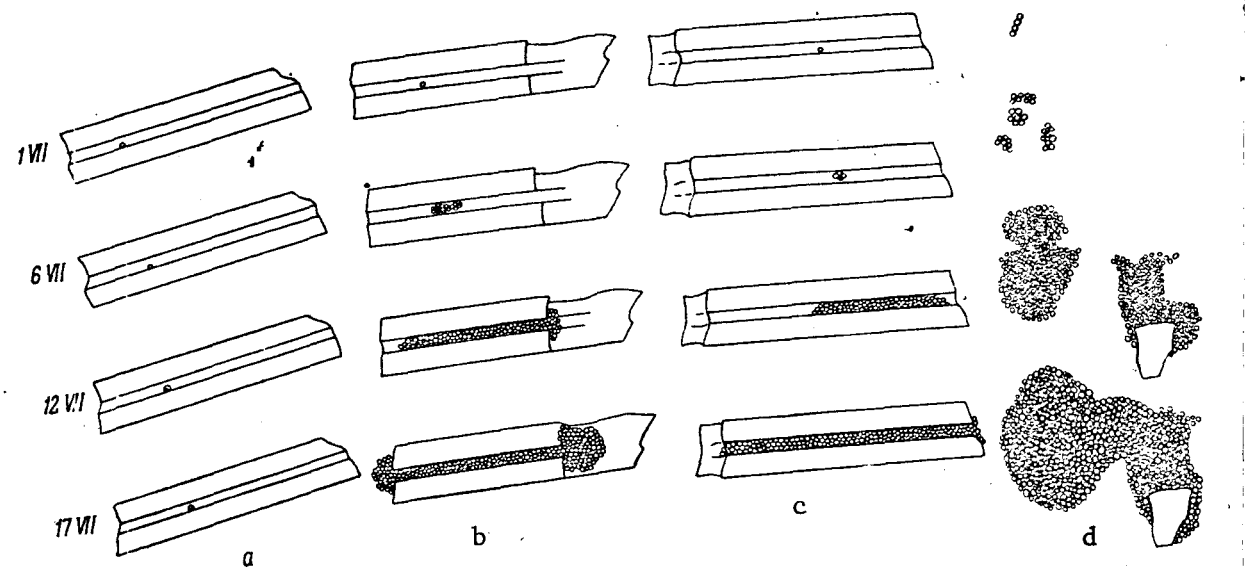

Figure 100

Microcultures in the Green Alga *Chlorella* Evolved from a Single Cell by Means of the Microselector.

a - Cell has been isolated, but has not proceeded to divide in the channel of the core fragment in all four periods of microculture observation; b, c - An isolated cell has proceeded to divide while remaining in the channel of the capillary core fragment; d - Immediately after placement of the capillary core fragment on the substrate, the isolated cell has left the channel; faster microcolony development is detected (magnification 10 x 45).

The described microcuvette device makes it possible to follow the germination of the isolated cell and its development under powerful microscopic magnification, while protected from external infection. Figure 99, for example, represents /216 germination of conidia of *Penicillium sp.* at room temperature on Czapek's agar on the second day after they were isolated with the capillary microselector.

Figure 100 shows the development of single-cell cultures of the green alga *Chlorella sp.* isolated by a microselector and sown in microcuvettes. Owing to the feasibility of employing strong magnification, we were able to trace the multiplication of this alga from a single cell. In one of the cases (a) shown in Figure 100, the isolated cell remaining in the channel did not even proceed to divide in 21 days of observation. In two cases (b, c) the original cell remained in the channel of the capillary core fragment. In the fourth case, the cell isolated in the core segment was soon carried out of the channel into the surrounding substrate (d). It had proceeded to divide a day earlier.

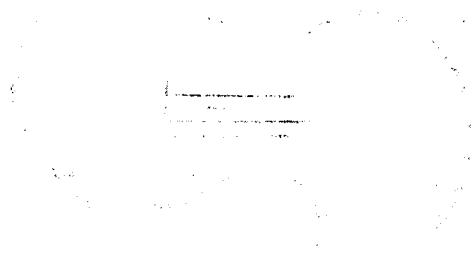

Figure 101

Microcolony of Day-old Diphtheria Bacillus Culture Evolved From a Single Cell Isolated By a Micro-Selector.

Staying in the channel apparently delays the cell-division rate, but does not introduce any essential disturbances. We ascertained this by observing the development of microcolonies of different microorganisms from cells which remained within the capillary core fragment. Such cases are rare during isolation by the microselector and are easily detected, since--if the isolated cell has remained in the channel--a typical biscuit-shaped (dumbbell-shaped) microcolony is formed when the cell divides repeatedly, as is evident in Figure 101. This figure represents a microphotograph of a day-old diphtheria bacillus microcolony grown from a single cell isolated by the capillary microselector (Bayard, 1951).

Only by means of the microcuvette may we trace the development of microbe cells when they are isolated by the microselector. During implantation in test tubes or flasks, we can derive an idea only of the percentage of successful isolations. The reasons for the failures, however, may vary: Either the segment of the capillary core with the cell did not reach the substrate during seeding, or possibly the isolated cell was non-viable and--although found on the substrate--did not proceed to divide. Cases are, moreover, not excluded /217
where the isolated cell begins to divide, but thereafter the development of the microcolony may be suppressed by some harmful factor, including cases of the multiplication of contaminating extraneous microflorae. And in fact, by using conidia of *Penicillium sp.* during isolation, we were able to determine more accurately the specific causes of negative results when working with the microselector. As is evident from Table 10, a certain number of detached core fragments with *Penicillium sp.* spores (about 20%) did not fall onto the substrate drops. The reason is that, when seeded, these fragments either appeared /218
in the microcuvette dispersed on the dry glass surface outside the drops--and therefore the cells in them could not develop--or they stuck to the surface of the capillary receiver and the cells died when the receiver was flame-sterilized.

It is natural that not every core segment with a cell gives rise to a microcolony after it has reached the substrate in the microcuvette since not all cells isolated are capable of germination or division. Owing, in fact, to the comparatively large size of *Penicillium* spores, we repeatedly had the experience of detecting cells in the core fragments which did not even germinate during the whole course of the experiment.

At times *Penicillium* conidia were absent from a detached fragment of the capillary core, whose channel was carefully examined at high magnification. Direct observations showed that the disappearance from the capillary channel of the isolated cell which is incapable of active movement, can occur only after it is immersed in the substrate drop, and is a purely physical phenomenon.

TABLE X

COMPUTED GERMINATION OF ISOLATED *PENICILLIUM* SPORES IN MICROCUVETTES

Order Number of Micro-cuvette	Number of Detach-ments Made	Number of Detached Segments Falling on Drops of Liquid Substrate	Number of Micro-colonies Developed From One Cell	Order Number of Micro-cuvette	Number of Detach-ments Made	Number of Detached Segments Falling on Drops of Liquid Substrate	Number of Micro-colonies Developed From One Cell
1	6	6	5	16	8	6	2
2	7	6	5	17	7	7	4
3	7	6	4	18	8	6	0
4	6	6	4	19	8	6	1
5	9	9	3	20	7	7	2
6	8	5	4	21	6	6	3
7	8	6	6	22	8	6	3
8	4	2	2	23	4	4	3
9	8	6	2	24	7	5	2
10	8	5	4	25	8	6	3
11	7	4	2	26	7	6	3
12	2	1	1	27	7	5	3
13	9	5	5	28	6	6	2
14	8	6	2	29	7	6	4
15	5	5	5	Total	205	160	89

The cells are carried out of the channel by the current of liquid generated by the difference in concentrations within and without the capillary. We conducted pertinent experiments confirming this assumption with the same spherical *Chlorella* cells which were very close in size (see Chapter 6, Section 5). It is difficult to find an individual cell in the microcuvette beyond the confines of the capillary.

It seems to us that use of the proposed microcuvettes could substantially advance the study of soil microflorae by Vinogradskiy's method in the versions of N. G. Kholodnyy (1936) and D. M. Novogrudskiy (1948). In the latter version, the essence of this method lies in the fact that fine earth obtained from soil reduced to an air-dry state, comminuted in a mortar, and sifted through a screen with a mesh of 0.25 mm, is sown on Petri dishes with agarized water. Because of the lack of any nutritive substances in the substrate, the microbes begin to develop in direct proximity to the soil particles. They multiply slowly, make no vast accumulations in the form of typical colonies, but live

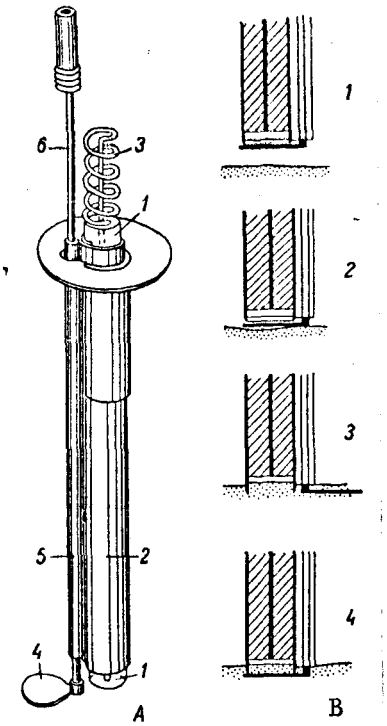

Figure 102

Instrument for Transferring Microcolonies From The Agar Medium Under the Microscope.

A - Construction of the instrument: 1 - Glass piston with longitudinal opening; 2 - Thin-walled tube with sharp edge of non-corrosive metal, 3 - Wire spring rod for putting the round cover glass on the colony, 4 - Rotating horizontal knife, 5, 6 - Handle of the rotating knife. B - Excision of colony from agar medium in Petri dish: 1, 2 -- Instrument with knife covering the tube opening is vertically placed at the surface of the agar over the colony, 3 - Knife is deflected to the side, and edges of tube are sunk in top layer of agar, 4 - Substrate over the colony is cut out by turning the knife.

incomparably longer than, for example, on meat-peptone agar. A growth picture is obtained which, in the opinion of D. M. Novogrudskiy, approaches that under natural circumstances, and is more suitable for protracted observations (Novogrudskiy, 1948).

During microscopy of the microcolonies, however, the Petri dish sown with fine earth must be open when placed under the microscope, since otherwise it is impossible to obtain the great magnification needed here. This undoubtedly leads to contamination from without, even of such a low-nutritive medium as agarized water, and to desiccation of this substrate. The considerable thickness of the agar layer, moreover, impairs the illumination conditions. Therefore, D. M. Novogrudskiy proposes that--for a more /219 detailed study of individual microcolonies of soil microflorae--individual small disks of agar be cut out of the Petri dish with a glass tube and be transferred to a separate glass. The latter operation may be rendered more precise if a special instrument designed by the authors is used. This instrument consists, as is shown in Figure 102A, of a thin-walled metal tube 8 to 10 mm in diameter with sharp edges of a non-corroding, biologically inert metal (tantalum or stainless steel) and a metal blade in the form of a thin disk of the same diameter as the tube and movably affixed to its lower part on a long rod. A cylindrical glass cylinder moves inside the metal tube. For every preparation, on the tube between the piston and knife we apply a changeable round covering glass.

In order to obtain a sufficiently thin slice of the selected superficial layer of agar, with minimum disturbance of the microscopic object, the instrument described (Figure 102B) with its lower opening closed and a circular

covering glass in place is brought close to the requisite location in the Petri dish. The cutting disk is diverted to the side by turning the handle, the edges of the cylinder are pressed into the agar to a slight depth, and the disk is returned to its original position. After that, a thin circular plate of agar with the object selected is cut off in the tube. In order to transfer it to the slide, it is sufficient to open the lower end of the tube in the immediate proximity of the slide surface by diverting the cutting disk to the side. Then the slice of agar with its little round covering glass falls onto the slide, particularly if the round covering glass is pressed slightly /220 by the wire pusher of the piston.

A clear picture, which is suitable for observations of great magnifications under the microscope, may, however, be obtained without disturbing microbe life around the fine earth particles if the fine earth is not sown in a Petri dish, but is sown directly in the above-described elongated, rectangular microcuvettes. If agarized water is applied in an uniform layer to the inner surface of the thin cover of the microcuvette, sown with fine earth, and if the microcuvette is placed in a Petri dish used as a humidity chamber--as described above (Figure 98)--the development of the soil microflorae, reliably protected against external infection and substrate desiccation (Table XIV, 1-3), may be traced repeatedly for a long time at high magnifications, including immersion.

To illustrate this, let us once more discuss an example of the use of microcuvettes. As previously reported (Chapter 2), we used a capillary peloscope to discover a large number of entirely new microorganisms. Most of them cannot be detected during inoculation onto the usual synthetic substrates. As it develops, however, some of these microbes are nevertheless capable of passing from the capillaries of the peloscope to agar media, at times becoming considerably modified morphologically. It is most convenient to use rectangular microcuvettes to follow the development of the microepibionts of the peloscopic cell or the fate of the strips placed on solid substrates of varying composition. Under high magnification, the content of the peloscopic cell channels and the microorganisms developing in the thin agar layer at the capillary outlets (Table XV) are clearly visible. Here, it must be borne in mind that by no means all microorganisms of loose soils are capable of producing colonies visible to the naked eye on a solid substrate. In some cases growth stops with the formation of individual microcolonies, which may be seen only under great magnification. Although occurring in considerable numbers, they are not visible in Petri dishes and are caught only in microcuvettes with careful examination under powerful magnification. Moreover, some typical representatives of mud microflorae--as, for example, *Metallogenium personatum* Perf--begin developing on an artificial substrate only after a protracted period of time (about 3 or 4 weeks) after inoculation.

For a microscopic study of anaerobes in microcultures, the capillary glass technique may provide a substantial supplement to the method proposed by Fortner (1935).

A clear picture of anaerobe development in the microculture, accesible to microscopy under powerful magnifications, may be obtained if germination is

carried on in a capillary with a rectangular channel and a thin flat lid. For purposes of strength, the bottom of such a capillary must be sufficiently heavy (about 1.0 mm). Capillary segments 70 to 100 mm long, with the transverse cross-section configuration shown in Figure 150, proved to be convenient for anaerobe microcultures. If a suspension of the proper dilution of the anaerobic culture in heated agar is drawn into such a capillary with a bulb, after which the ends of the capillary are plugged with cement (Mendeleyev or Krengel), anaerobic conditions are created in the channel without any additional oxygen absorbents. In contrast to the Vignal-Veyon method based on the use of round glass tubes, the consecutive development of microcolonies and individual microbe cells can be clearly examined microscopically under high magnification, including immersion, in a rectangular glass capillary with a flat lid and with slight channel depth.

If necessary, selected microcolonies may be excised from the capillary microcuvette for further propagation in pure cultures. Because of the simple configuration of their cross-section and their slight thickness, these capillaries are inexpensive when manufactured in great quantity by drawing.

Table XIV, 4 and 5, show microphotographs of a bacterium similar to *Clostridium pectinovorum* developing in a capillary channel. The photos of the living culture were made under low magnification and in oil immersion.

MANUFACTURE OF THIN GLASS STRIPS, PLATES, AND COVER GLASSES BY DRAWING SHEET GLASS

Heretofore the problem of the industrial manufacture of inexpensive and /463
high-grade microchambers and microcuvettes needed for studying the growth and development of microorganisms under the microscope at high magnifications, and for various other experimental operations, has remained practically unsolved.

The demand for thin glass plates of a given thickness in different fields of industry and experimental physics is far from being satisfied.

With the continuously growing volume of scientific and practical work connected with microscopy, the lack of cover glasses, which are needed not only in standard size, but also in the form of more or less long strips, is being more and more manifested. The demand for high-grade cover glasses suitable for important work under the very highest microscopic magnification is especially great. In this case, the thickness of the cover glass must not exceed 0.15-0.17/mm. It is our opinion that comparatively narrow ribbon-shaped cover glasses are advisable for mounting microtomic series.

The reason for the extreme short-supply of high-grade cover glasses and lack of production of glass strips and various microcuvettes and microchambers suitable for microscopy under high magnifications is the extremely low techno- /464
logical level of the manufacture of especially thin glass--in particular, cover glasses for microscopy.

Even in the Soviet Union, at the same time that microscopes are being continuously and successfully improved, cover glasses are being made by the old-fashioned method of hand-blowing which has come down in almost unchanged form from the Middle Ages (Gastev and Rodin, 1946; Lebedev, 1928).

The manufacture of cover glasses by the hand-blowing method, as is well known, is as follows. The master glassblower picks up a small dollop of the glass mass on the previously heated end of the pipe. The bit of glass is given an egg-shape and the so-called "flask" or "gather" is obtained. Then the master collects the needed amount of glass mass on the flask, rolls it on the wooden marver, and proceeds to blow the glass, gradually rotating the pipe about its longitudinal axis and blowing in air by mouth. The result is that the gathered portion of the glass mass is blown out by the master into a big bubble of elongated shape. The final shaping is given to the blown glass in the so-called "pit", a deep trench in which the master swings the blown glass like a pendulum while continuously blowing air into the pipe. After blowing the glass out to a certain size, the master detaches it from the pipe with a slight tap. Grasping the blown glass with special tongs the assisting journeyman transfers it to the annealing furnace. After annealing, the top and bottom of the blown glass are cut off, converting it into a cylinder. The cylinder is cut with a diamond along the generatrix and is converted into a sheet in special separating or melting furnaces. In the cutting shop, the sheet is cut into pieces of the proper size. Before this, it is once more

annealed.

The hand-blowing method of manufacturing sheet glass requires great skill and exertion of physical strength from the worker, and at the same time it is of low efficiency. It is attended with great waste since only a small portion of the blown cylinder attains the requisite thickness with an even surface. The industrial production of window and other sheet glass has been entirely converted to the machine method, whose efficiency is continuously growing. The machines used for this purpose, however, are capable of processing sheet glass no thinner than 1 mm or, as an exception, 0.8 mm, and this size is attainable by only a few plants. At present, in the city of Klin a pilot installation for drawing sheet glass 0.5 to 0.6 mm thick of a milky color from the glass mass has been put into operation (Tsaritsyn, 1960). In the production of cover glasses answering the requirements of modern microscopy, the mechanized process is not used at all. The glass factory "Druzhwava Gorka" is presently performing experiments on producing cover glass by a mechanized method. But inasmuch as the strip is drawn from a molten glass mass, the quality of cover glasses is reduced because of banding (Tsaritsyn, 1960). This author reports that in Germany the transition is being made to mechanized manufacture of cover glasses made of sheet glass.

Using the "principle of similitude drawing" earlier examined, since 1949 we have been manufacturing in the laboratory especially thin glass plates with a thickness of 1.0 to 2.0 mm (from photographic glass). According to this principle, a wide glass plate of slight thickness should, when drawn, be reduced in both its cross-sectional dimensions by the same factor n and be converted into /465 a thin flat ribbon.

If, for example, the initial plate is 100 mm wide, 1.2 mm thick, and has an effective length of 100 mm, then to produce a ribbon 0.17 mm thick the width of the glass work-piece must be reduced by a factor of six since 1.2:0.17 = 6. The width of the ribbon obtained will be about 17 mm (100:6). The expected length of such a ribbon may be found from the above formula: $y = 100 \times 6^2 = 3600$ mm or 3.6 m.

To maintain the configuration of the plate while being drawn, in addition to a specific temperature regime, a special method of fastening the plates to prevent them from bending and deforming is needed. This problem is solved by fastening the initial glass plate work-piece in a pair of flat and elastic holders, each of which may consist of two layers of asbestos board immediately contiguous to the glass, and two iron plates clamping the glass to the asbestos by means of metal cleats and bolts. The asbestos board may be replaced by plates of transformer iron.

Efficiency may be sharply increased by using a method of simultaneous drawing of several work-pieces in one furnace to produce glass strips (see Section 1), in this case raising the number of plates to be drawn to 5 to 7. It is only necessary that each of the glass plates be securely compressed and does not touch the adjacent plates during the drawing process to avoid deformation and, especially, sticking. This requirement is easy to fulfill in

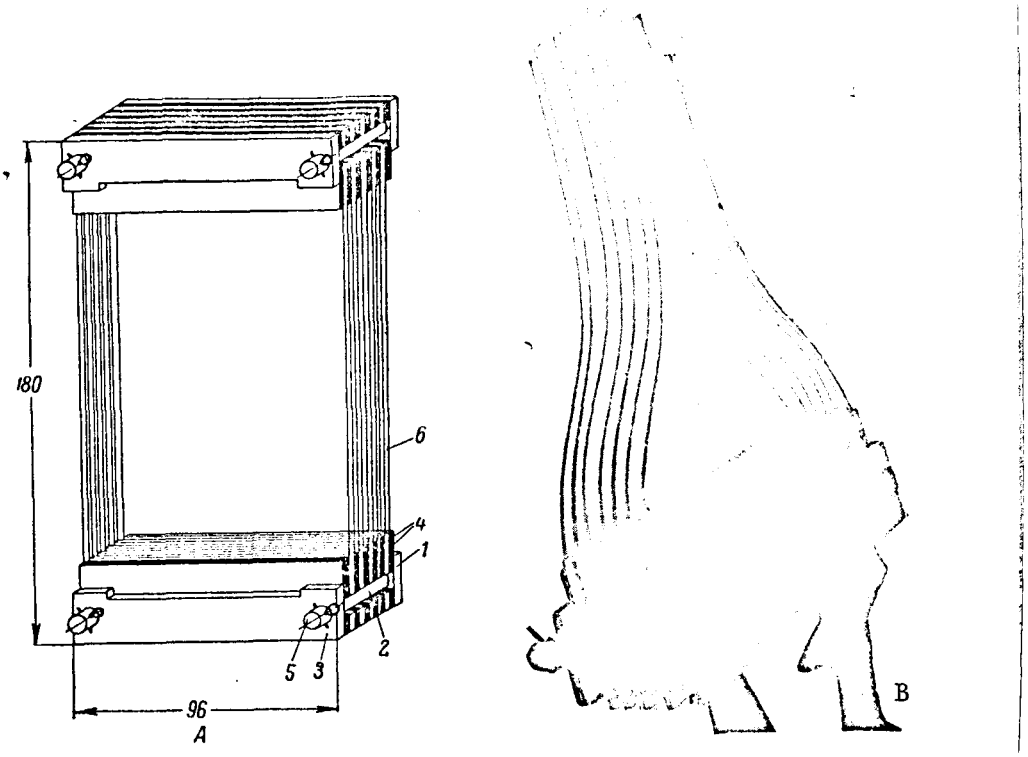

Figure 236

Stack of Seven Glass Plates with Side Notches Assembled With a Pair of Holders.

A - Before drawing: 1 - Rear holder cleat, 2 - Pin, 3 - Front cleat, 4 - Spacers, 5 - Cotter pin, 6 - Glass plates. B - View of one holder with remains of glass plates after drawing.

practice by gathering the plates into a block with a simple attachment consisting of two pairs of metal cleats and a set of thin spacers of transformer iron. By means of lateral notches, each of these iron spacers is held on the pins of the fastening cleats (Fig. 236).

In order that the stack of glass plates will be more solidly fastened in the two holders, on the upper and lower ends of each of the plates paired lateral grooves may be made with a diamond file.

Such a block is assembled as follows. The upper and lower fastening cleats with their pins (threaded at the ends) are placed on a horizontal surface (e.g., a table) with the pins upwards, and first a layer of four or five iron spacers is placed on each of them, and then a glass plate. We separate the second glass plate from the first with a new layer of iron plates, and so on. On the last glass plate we place the last layer of spacers and tighten the whole block with a couple of auxiliary cleats and cotter pins, or better still, nuts (Fig. 236A).

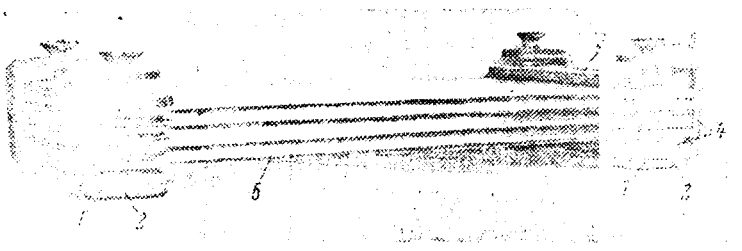

Figure 237

Version of Holder for Glass Plates Which Does Not Require Grooves in Plates.

1 - Immobile cleat connecting the two U-shaped clamps, 2 - Holder, 3 - Removable cleat, 4 - Spacers of transformer iron, 5 - Clamping screws, 6 - Glass plate work-pieces.

The essential disadvantage of this method of assembling the block is that the grooves may be made in the glass plates only with a diamond file in good working condition. This operation is rather laborious and may lead to crack formation in the glass plates, which are particularly dangerous if they remain hidden during assembly of the block and are discovered only in stretching.

Therefore, as a variant we utilize a holder which is suited to clamping the plates without grooves. In this second method, the preparatory work is substantially curtailed and the danger of latent cracks in the plate work-pieces is reduced. A block of these plates, however, requires more care during preliminary heating since with an inadequately tightened clamp the block may come apart, and with too great tightening of the holder the individual plates may /466 crack.

Best results are obtained with spacers of asbestos board. In this case, because of the relative softness of the material, the glass plates are not destroyed even if they are compressed in the block with greater force than in the preceding version. The second advantage of asbestos is that it does not bake into the glass, even with considerable overheating. But because of the substantial thickness of the asbestos board, no more than four or five glass plates may be fastened in a holder.

The holder design represented in Fig. 237 proved to be of practical use. The width of such a holder is about 90 mm, but the glass plates may be wider. The spacers, as in the first version, are made of transformer iron of the same width (90 mm), but without notches, or they are made of asbestos board. One of the cleats of each holder is solidly fastened to a pair of U-shaped clamps and joins them (2). The second cleat (3) of thick sheet iron (2 - 3 mm) is pressed to the glass (through iron spacers) by a pair of short screws (5). In order

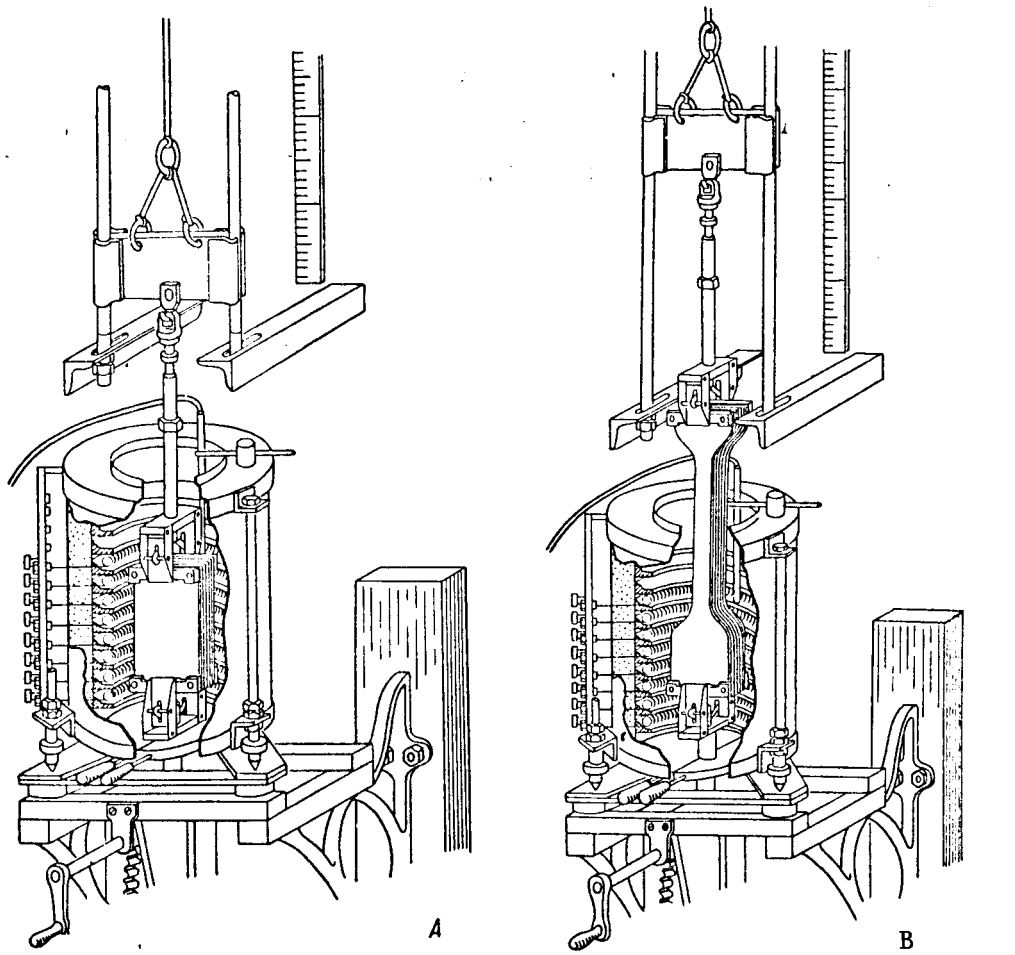

Figure 238

Holder With Stack of Glass Plates in Sectional Shaft Furnace.

A - During preliminary heating; B - At Start of drawing.

that this holder may be held more securely in the furnace clamps, the external sides of all four of its metal cleats each have a shallow longitudinal groove.

The block of glass plates assembled in one manner or another is then placed in the earlier described sectional shaft furnace (Chapter 14, Section 3) for drawing. The block is held here in a vertical position by the upper and lower pincers of the device. /467

During the preliminary heating, the block occupies the lower position, as shown in Fig. 238. Here the upper holder with a plate length of 18 cm must be

above the maximum temperature zone. As the temperature rises, the upper and lower pincers must be periodically carefully tightened. In the case of transformer iron spacers, care must be taken that the neck formed when the plates have begun to be drawn apart is located considerably below the upper holder. Otherwise, the iron plates may bake into the glass, which, when the holder cools as it leaves the furnace, results in cracking of the upper ends of the strips and in their tearing away from the upper holder.

In contrast to the drawing of individual work-pieces, the tension in this case may not be interrupted even for a short period; otherwise, the individual glass plates in the softening zone will unavoidably stick together. Therefore, it is necessary to take care that the motion of the upper pincers is not stopped by the top baffle of the furnace (which must be opened in good time) or by any other obstacle. Possible bending or sticking of the softened plates is also prevented by applying sufficient tensile force to the block (not less than 0.9 to 1.3 kg).

The conversion of plates into strips is shown in Fig. 238,B, while Fig. 236,B represents the lower holder with the remains of the glass plates after drawing.

The result is to produce thin glass strips of plane-parallel cross-section of given thickness, basically corresponding to the calculations. A certain deviation from the original form is apparent in the slight thickening of the edges of the strip because of nonuniform temperature distribution in the softening zone of the workpiece. In the central part of the shaft, the temperature is somewhat lower than in the immediate neighborhood of the heating elements (as has already been mentioned in Chapter 14). We assume that by converting to a shaft furnace of large diameter this defect may be completely corrected. As tests have shown, these strips, even with thickened edges, are /468 quite suited for microscopy of the object at various magnifications, including immersion in oil.

The same reason--i.e., nonuniform furnace temperature field within each zone when the glass plates are of great width--apparently also causes a certain disparity in the width of adjacent segments of the parallel strips. However, despite the perceptible fluctuations in width, the greater part of the drawn article--above 90%--proved to be within the given size limits.

The glass strips assorted by thickness may be cut with a diamond pencil into square segments of proper size for cover glasses. It is convenient to make cover glasses with ground edges from the strips. For this purpose, a block of 60 to 90 strip segments of about equal width and each 100 to 150 mm long is assembled in Krengel' s cement (Fig. 239a). On a polishing machine, a /469 block of these strips is ground together to the requisite width -- i.e., to 15-18 mm and a second pair of protective plates is cemented on the ground surfaces (Fig. 239b) The block thus prepared is cut with a diamond saw into four to seven segments, depending on the prescribed length of the cover glasses (Fig. 239c).

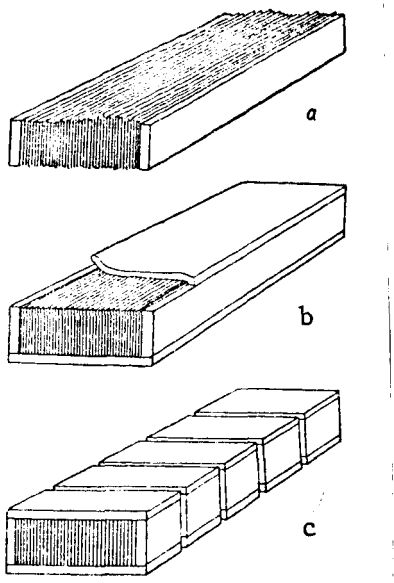

Figure 239

Preparation of Cover Glasses Made of Glass Strips

a - Stack of glass strips is cemented to the block between a pair of protective side plates; b - The second pair of protective plates is placed upon the block (upper and lower); c - The block is cut with a diamond saw.

The following calculations may give an idea of the efficiency of our proposed method of manufacturing cover glasses. As indicated above, from one plate of sheet glass 100 mm wide, 1.2 mm thick, and length (effective) of 100 mm we may obtain a strip which--with a thickness of 0.17 mm and a corresponding width of 17 mm--will have a length of 3.6 m. When seven plates are simultaneously drawn, the total strip length will reach 3.6 x 7 = 25.2 m. As the result of a single drawing lasting 3 to 4 hours, including preliminary heating, we may obtain more than 20 m of glass strip which will yield up to 1000 cover glasses. The same strips cut into longer pieces are the initial material for the manufacture of microcuvettes (see Chapter 8) and a different type of flow-through chambers (Chapters 9 and 10).

To summarize, we may state the following:

1. The method which we propose for manufacturing especially thin glass plates and cover glasses requires no special room or expensive equipment. All the equipment needed may be restricted to a drawing installation of relatively simple construction, a grinding machine, and a diamond saw (the latter is not obligatory if plans do not include manufacturing cover glasses with edges ground

to the same width). The work may be performed in a small room with even room temperature and sufficiently high ceiling (not lower than 3.5 - 4.0 meters).

2. No great skill or specialization is required of the operator to perform this work. The work involves no physical exertion.

3. The new method makes it possible to produce a thin flat glass strip in a considerable choice of width and thickness based on a simple preliminary calculation using the principle of "similitude drawing."

4. The glass strips thus produced are entirely satisfactory for microscopic work; in comparison to hand-blowing, the loss is insignificant.

5. Because of the feasibility of drawing several strips at once on one device, the proposed method is distinguished by its adequately high efficiency.

6. Since it is based on the use of measurable and regulatable factors /470 (like furnace temperature regime, rate of block feed into the softening zone, degree of tensile force), the method which we propose permits mechanization and automation of the drawing process. This leads to the feasibility of converting the very backward business of manufacturing especially thin glass plates needed for cover glasses, microcuvettes, microchambers, etc. to a profitable machine technique.

Scientific Translation Service
4849 Tocaloma Lane
La Canada, California